as negative controls. A secondary anti-guinea pig lgG(H+L) HRP (diluted 1:1000) (KPL Gaithersburg, Md.) was used as a detection antibody. 3,3',5,5'-Tetramethylbenzidine (TMB) (KPL Gaithersburg, Md.) was used as a substrate and plates were allowed to develop for 20 minutes until TMB stop solution (KPL Gaithersburg, Md.) was added. Antibody titer was determined as the point when mean OD is twice above background OD (normal guinea pig sera).

[0162] Antibody mediated complement lysis assay. Vero cells were infected with Venezuelan equine encephalitis virus (VEEV) replicons expressing GP from either EBOV strain Zaire or MARV strain Musoke at moi 20. Following 16 hours of incubation, cells were removed and labeled with ^{51}Cr for 1 hour. Antibodies were diluted from 1:10 to 1:1280 and mixed with ^{51}Cr labeled, GP expressing vero cells. Lastly, guinea pig complement (Cedar Lane Laboratories, Hornby, Ontario, Canada) was added at a final dilution of 1:30. Each plate was then incubated for 3 hours at 37° C. All cell supernatants were transferred to luma plates and later read on a gamma counter. Antibodies titers capable of lysing GP-expressing vero cells in the presence of complement was determined as being the last dilution were gamma counts where twice above background levels generated from normal guinea pig sera.

[0163] EBOV and MARV Neutralization Assays. Guinea pig serum was diluted 1:10 to 1:160 in EMEM+10% FBS. EBOV strain Zaire or MARV strain Musoke viral stocks were diluted to 1000 pfu/ml in 20% guinea pig complement in EMEM. Serum dilutions and virus were then mixed and incubated for 1 hour at 37° C. A 100 uL mixture of the virus and serum was added to 6-well plates with a confluent layer of vero cells and incubated for 1 hour. A primary 1% agarose overlay was added and the plates were incubated at 37° C. for 7 days. After 7 days, a secondary 1% agarose overlay, containing 5% neutral red, was added and plaques were counted the following day. Neutralization titer was recorded as the point where the serum dilution resulted in an average of greater than 80% plaque reduction between each duplicate well.

[0164] Statistical Analysis. Antibody, neutralizing, and complement fixing titers were analyzed for significance between vaccination groups by Kruskal-Wallis one-way analysis of variance on ranks. Multiple comparisons between groups were evaluated by the Student-Newman-Keuls Method or Dunn's tests where appropriate. Survival LogRank tests were conducted to determine overall significance among all experimental groups analyzed. Multiple comparisons, for each treatment group, were done pairwise using the Holm-Sidak method.

Results

[0165] Characterization of MARV and EBOV Chimeric VLPs. VLPs expressing chimeric GP were separated by SDS PAGE, blotted onto PVDF membranes, and probed with MARV and EBOV GP1 and GP2 specific antibodies. We found each chimeric VLP preparation was a hybrid of both MARV and EBOV GP (data not shown). Both MARVGP1/EBOVGP2 and EBOVGP1/MARVGP2 separated between 90-120 kDa. EBOV GP1/2 and MARV GP1/2 VLPs separated similarly. We did not see any reactivity, using EBOV or MARV specific GP1 or GP2 antibodies, to GP subunits not engineered into the chimeric VLPs. Individual Ebola VLPs contained glycoproteins from GP1 of MARV and GP2 of EBOV or GP1 from EBOV and GP2 from MARV by EM (data not shown). Dual staining for GP1 and GP2, using different size gold beads for each marker, showed surface distribution of each viruses' GP on VLPs. Chimeric GP particles morphologically were similar to Ebola VLPs with unaltered GP (data not shown).

[0166] Antibody Response to MARV/EBOV Chimeric VLPs. After 2 immunizations with chimeric MARVGP1/EBOVGP2 VLPs the average MARV specific titer (IgG heavy and light chains) was 1000, and 400 for EBOV GP1/MARVGP2 VLP vaccinated guinea pigs (see FIG. **23**A). One way ANOVA revealed significant differences between vaccination groups ($p<0.001$). All vaccination groups produced significantly higher MARV antibody titers than the saline group ($p<0.05$). EBOV antibody titers were, on average, higher than MARV antibody titers. The mean EBOV titer for the MARVGP1/EBOVGP2 VLP vaccinated group was 7705 and 3960 for the EBOVGP1/MARVGP2 VLP vaccinated group (see FIG. **23**B). All vaccinated groups produced significantly higher EBOV antibody titers ($p<0.05$) than saline immunized controls. All MARV GP1/2 VLP vaccinated guinea pigs had MARV specific antibody titers greater than 2560, and EBOV GP1/2 vaccinated guinea pigs had EBOV specific titers greater than 10240 (see FIGS. **23**A and B). All guinea pigs vaccinated with saline did not produce detectible antibody titers to MARV or EBOV. In addition, animals vaccinated with MARV VLPs did not make antibodies to EBOV proteins, and EBOV VLP vaccinated animals did not produce antibodies that reacted to MARV proteins (data not shown).

[0167] Functional antibody titers. Sera, from vaccinated guinea pigs, were tested for neutralizing and complement fixing antibodies to EBOV or MARV GP. MARVGP1/EBOVGP2 and EBOVGP1/MARVGP2 VLP vaccinated guinea pigs' average MARV complement fixing antibody titers were 1:65 and 1:55 respectively (see FIG. **24**A). Both of which were significantly higher than titers observed for saline vaccinated controls ($p<0.05$). EBOV complement fixing antibody titers were much higher in both groups. MARVGP1/EBOVGP2 and EBOVGP1/MARVGP2 VLP vaccinated guinea pigs' average EBOV complement fixing antibody titers were greater than 1:1280 and 1:660 respectively (see FIG. **24**B). These were also significantly higher than titers from saline vaccinated controls ($p<0.05$). All MARV GP1/2 VLP vaccinated guinea pigs had complement fixing antibody titers greater than or equal to 1:40, and all EBOV GP1/2 vaccinated guinea pigs had titers greater than or equal to 1:1280. Vaccination with chimeric VLPs resulted in MARV and EBOV neutralizing antibodies. MARVGP1/EBOVGP2 and EBOVGP1/MARVGP2 VLP vaccinated guinea pigs' average MARV neutralizing titer was 1:67 and 1:86 respectively (see FIG. **25**A). Both groups had significantly higher levels of neutralizing antibodies than saline vaccinated controls ($p>0.05$). Similar to EBOV antibody and complement fixing titers, EBOV neutralizing titers were nearly 3 times, on average, higher than MARV neutralizing titers. MARVGP1/EBOVGP2 and EBOVGP1/MARVGP2 VLP vaccinated guinea pigs' average EBOV neutralizing titer was 1:302 and 1:50 respectively (see FIG. **25**B). EBOVGP1/MARVGP2 VLP vaccinated animals did not produce significantly higher ($p>0.05$) neutralizing titers than saline immunized controls; however, MARVGP1/EBOVGP2 vaccinated guinea pigs produced significantly higher neutralization titers than saline immunized controls ($p>0.05$). All MARV GP1/2 VLP vaccinated guinea pigs generated MARV antibody neutralizing titers greater than or equal to 1:160, and EBOV GP1/2 VLP vaccinated guinea pigs' average EBOV neutralizing titers